Art. XXVIII.—*Enteritis, Associated with Infection of the Intestinal Wall by Cyst-forming Protozoa (Neosporidia), Occurring in certain Native Animals (Wallaby, Kangaroo and Wombat).*

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(With Plates LXXII-LXXXI.).

[Read 14th December, 1911].

For some considerable period post-mortem examinations have been made at the University Veterinary Institute of the majority of native animals which have died at the Zoological Gardens, Melbourne. Quite recently several cases of more or less acute enteritis, evidently the immediate cause of death, have been observed, and in each of these extensive invasion of the mucosa or submucosa by protozoa has been detected on microscopical examination.

As will be seen, the protozoan infection in all cases, with the exception of one wallaby, was very extensive, and must have been in existence for a considerable period prior to death. Obviously, therefore, to this infection the acute inflammation cannot be entirely attributed. It is especially worthy of note that in every instance the animal was a recent arrival at the Zoological Gardens, having been captured, conveyed to Melbourne, and delivered apparently in normal health. Probably the confinement, coupled with the drastic change of food and environment, enabled bacteria to invade the damaged and weakened intestinal wall, and so established the acute enteritis, the immediate cause of death, for even in those cases where post-

mortem examination was made a few hours after death, extensive bacterial invasion of the intestinal coats was very definite. It may be that more extended investigation of further cases will demonstrate that these parasites which at present appear to be of four distinct species, possibly a new genus, are but the one species varying according to the animal and according to the cells invaded. The terms applied to these protozoa in this article are therefore naturally provisional, and in the absence of full knowledge of their life history are only employed to avoid confusion and repetition in future references.

So far as we are aware, such parasites have not previously been recorded, though that referred to as *Sarcocystis mucosa*, Blanchard, by Ray Lankester (Treatise on Zoology, Part I., p. 351), and by Guiart (Parasitologie, p. 239), who includes it with the sub-genus *Balbiania*, may prove on consulting Blanchard's original memoir, which at present is not available, to be the same as one or other of the forms we describe.

Sarcocystis macropodis from Wallaby (Petrogale sp.).

The protozoan infection has been observed in three animals. In two cases the immediate cause of death was acute enteritis, affecting the greater part of the small intestine in each, the parasitic cyst being confined to the inflamed portions, and present in enormous numbers, but in the other specimen there was no acute inflammation, while the cysts were very few in number, not more than a dozen being present in the whole of the intestinal wall. In this note those manifesting the acute enteritis are alone dealt with.

On post-mortem examination the whole of the small intestine was observed to be mottled with irregular areas of deep congestion, visible from the serous surface. On closer examination these areas were seen to be composed of groups of fairly regular circular nodules with pale centre surrounded by a deeply congested periphery. The whole mucosa on incision was found to be catarrhal and deeply congested, especially around and above the small nodules, which distinctly projected into the lumen as dark red elevations each about the average size of a grain of mustard. The stomach and large intestines were normal, as were the other

viscera, with the exception of one mesenteric lymph gland, which showed enlargement and hyperaemia, and on section was found to be mottled with numerous circular greyish areas, pinhead or smaller in diameter, due to proliferation of groups of fixed cells surrounded by a zone of closely packed lymphocytes.

The small intestinal nodules could be removed from the submucosa in which they were situated with some difficulty. They then presented a translucent cystic appearance, and could be readily squeezed and ruptured between two slides. Examination of the smears made in this fashion demonstrated the presence of spindle-shaped nucleated sporozoites, similar in many respects to the spores of sarcosporidia.

In sections of the intestines examined under the low power of the microscope, the submucosa is seen to be greatly thickened by spherical masses of new, loose, fibrous tissue enclosing distinct parasitic cysts (Plate LXXII., Figs. 1 and 2), which are so numerous that in many parts a single field shows from ten to twelve. These cysts, together with the surrounding inflammatory tissue, may be found singly or clustered together, in some cases the mucosa being so implicated as to result in complete atrophy of the muscularis mucosa (Plate LXXIII., Figs. 3 and 4). The periphery of the new inflammatory tissue is extremely vascular, the capillaries being greatly distended with blood, and haemorrhages are frequent. In certain regions, especially where the cysts are numerous and close together, there is atrophy of Lieberkühn's glands, and even complete disappearance of many villi. The mucosa is congested, and there is complete denudation of the surface epithelium, even in the specimens fixed very soon after death of the animal.

The parasitic cysts vary considerably in size, generally according to the stage of development: the younger stages observed vary from 0.15 mm. to 0.26 mm.; the intermediate stage from 0.3 mm. to 0.5 mm.; and the mature cysts from 0.36 mm. to 0.7 mm. in diameter. They are roughly spherical as a rule, but individual specimens in which there is considerable distortion are not uncommon.

For purposes of description the parasite may be divided into envelope and contents.

The envelope consists of two zones, an external radiate and fringe-like, and an internal on which the fringes of the outer

zone rest, uniform in thickness and staining deeply. The external "fringe" is fairly uniform in breadth, averaging 20 μ in the younger stages (Plate LXXIV., Fig. 2), but in the older stages it is extremely irregular, especially at certain situations where it sends out long branching and anastomosing processes into the surrounding tissue, and is invaded by polymorphs (Plate LXXIV., Fig. 6), of which, immediately around nearly all cysts, there is a distinct accumulation.

The contents consist of a homogeneous endoplasm, uniformly staining, but for faint concentric striations probably due to contraction in fixing. Towards the centre of this endoplasm are situated numerous blastophores, more or less spherical areas without a limiting membrane containing nuclei, which gradually develop into mature spores. The younger the parasite the greater is the amount of endoplasm present, and in the earliest stages observed it not only distinctly separates each blastophore from its neighbour, but more completely separates the most peripheral of the blastophores from its envelope. As the cyst develops, however, the endoplasm becomes reduced, ultimately the blastophores generally, though not always, lying close together, and immediately underneath the inner zone of the envelope (Plate LXXV., Fig. 7). Both the envelope and the endoplasm stain definitely with eosin, and such acidophile dyes.

The blastophores within the endoplasm vary considerably in number, the average being from 60 to 80 in a median section. What may be described as three distinct stages are observed. In the youngest cysts (measuring from 0.0134 mm. to 0.0335 mm. in diameter) they are irregularly but more or less centrally grouped. The nuclei, which vary from 7 to 20, are peripherally arranged, and lie in a fine granular basophile material. The second stage shows an increase both in size (0.225 to 0.0584 mm.) and in the number of nuclei, the granular material being separated into masses around which the nuclei are arranged. These masses seem to divide as the nuclei further increase and ultimately each blastophore is crowded with nuclei still arranged mulberry-like around small portions of the granular material. In the third stage (0.0334 to 0.075 mm.) of the development, the nuclei are seen to develop small peripheral cones of finely granular protoplasm; gradually the central mass divides until

in this manner fully-developed spores are formed, these being crowded together within the enlarged blastophore (Plate LXXV., Fig. 7), but still in most cases grouped radially around a varying quantity of residual protoplasm. In some sections free spores may be seen exterior to the cysts, these having escaped through the now thin and ruptured envelope.

Degeneration of the Blastophores.

What is apparently a degeneration of some of the blastophores is of frequent occurrence, for in all stages of development of the cysts one or more spaces may be seen which correspond to the blastophores, but are generally much larger and usually situated at or near the centre of the cyst. These spaces contain a loose basophile granular material, evidently the chromatin of broken-down nuclei. Different phases of the nuclear disintegration may be seen in various cysts.

Mature spores.

These are best examined in smears prepared by squeezing the contents of the cysts between a coverglass and slide, fixing in alcohol, and staining by giemsa or iron haematoxylin and eosin. Spores thus fixed and stained are found to be ovoid or falciform bodies narrowing towards one end, and measuring 7.3μ to 12.4μ long by 3.4μ to 4.9μ broad. A large nucleus situated rather nearer the narrower extremity than the centre. In no instance does the nucleus stain deeply, and often it is less definitely stained than the cytoplasm, appearing as a somewhat clear ovoid space of nearly the same diameter as the spore. Scattered throughout the cytoplasm are numerous granules situated chiefly towards the blunter and broader end.

Ileocystis macropodis and Lymphocystis macropodis in Kangaroo (Macropus sp.).

The infection has been observed in only one specimen, a young, half-grown kangaroo, which died a few days after arrival

from Gippsland, where it had been captured. The carcase showed evidence of very acute diarrhoea, indeed dysentery, having been present prior to death. Post-mortem examination showed intense haemorrhagic inflammation of the greater part of the intestines, commencing about eighteen inches from the pylorus, as small and scattered petechiae, gradually becoming more numerous and ultimately confluent further along the canal. The caecum was but slightly affected, and the inflammation gradually disappeared in the large intestine, the rectum being normal. The intestinal contents were fluid, and mixed with much blood, the result of numerous haemorrhages. The inflammation, though chiefly confined to the mucosa, yet implicated to some extent all the coats of the viscus. From the serous surface no nodular appearance was visible, but on washing the intensely catarrhal mucosa, it was seen to be affected with numerous irregular greyish granular elevations. The mesenteric lymph glands were all oedematous and haemorrhagic, especially the colic gland, which was greatly enlarged and intensely haemorrhagic throughout.

Smears of the intestinal contents showed on microscopical examination innumerable falciform bodies (similar to those of sarcosporidia) to be described later.

Sections of the intestine examined microscopically show great distension of the smaller blood vessels, especially of the mucosa and very frequent haemorrhages. The villi, and indeed the whole mucosa, are much swollen, and close examination demonstrates the presence of two forms of parasitic cysts, some very large and closely packed in groups, and others very numerous, much smaller, and loosely scattered. There is but little tissue reaction, but the larger cysts are generally surrounded by a zone of leucocytes, and some new inflammatory tissue, though small in amount. The larger cysts are almost confined to the deeper areas of the mucosa (Plate LXVII., Fig. 11), but the small "cysts" are distributed fairly uniformly throughout the mucosa, even almost to the apices of the villi, often over considerable areas being entirely unassociated with the large cysts. As these varieties of "cysts" differ in many respects they must be described separately.

Large Cysts. Ileocystis macropodis.

These may be found singly, but generally in groups or clusters of three to eight (Plate LXXVI., Figs. 2 and 10). At times irregular in size, they are generally fairly uniform, when the appearance is that of a circular mass divided distinctly into three or more triangular segments, each with a crescentic base. These groups vary in size from 0.07 to 0.334 mm. in diameter. Like the wallaby cysts, each is provided with an envelope and with a homogeneous endoplasm within which is situated one blastophore (or perhaps schizont). The envelope shows at the free edge a fringe-like external coat, of an average breadth of 8μ, the striations being much finer and more regular than in the wallaby cysts. There is a definite thin inner coat, staining faintly blue with haematoxylin and eosin, and brownish with Van Gieson, while the bases of the fringe form at their junction a faintly staining intermediate zone, into which the striations are seen to prolong. The fringe and the endoplasm are acidophile, the former staining the less deeply. Where two cysts approximate the fringe is lost, but the faintly staining intermediate zone can be seen to extend between the two adjacent cysts, separating the endoplasm of the one from the other, and varying in breadth in the different groups (Plate LXXVIII., Fig. 12).

Within the endoplasm, lying almost invariably next the crescentic free border, lies always (as can be seen in serial sections) a large granular deeply staining body, which varies in shape: in the smaller cysts it is irregularly spherical, and exceeds in size the blastophore, in the larger cysts it is in the form of a long triangle or narrow long spindle (Plate LXXVI., Figs. 9 and 10). This body is probably the hypertrophied nucleus of the original cell, and indeed what appear to be the large spherical nucleoli can often be detected among the deeply staining granular material. These nuclei vary in size greatly, being in the younger cysts 14.6μ in diameter, and in the oldest from 79.2μ to 95.9μ long, and 8.34μ to 18.7μ broad. At first we were inclined to the opinion that these peripheral deeply staining masses were degenerated blastophores similar to those seen in

the wallaby, but the fact that in serial sections each cyst is found to be definitely associated with one of these masses, and that they are always in the same situation negatives such a view.

In the early stages the blastophore or schizont appears as a faintly staining space (12.8μ to 15μ in diameter) crowded with nuclei (1.7μ in diameter), and surrounded by endoplasm. As the parasite increases in size these nuclei multiply greatly, the cell nucleus becoming definitely pushed to the pole furthest from the centre of the group. The nuclei of the parasite are at times seen arranged around a central mass mulberry fashion, but often no such arrangement is distinct. Gradually they become surrounded by a spindle-shaped mass of faintly-staining protoplasm with blunt ends, and no definite markings, attaining in size 4.3μ to 4.7μ long, by 1.7μ to 1.9μ broad. In none of our sections are the spores of these large cysts seen to be mature, and there is no evidence of rupture.

Small Cysts. Lymphocystis macropodis.

(Although for purposes of description these are here so termed, we recognise that the parasites in question cannot, strictly speaking, be classed as "cysts.")

All the villi in our sections, as already stated, are greatly swollen, and almost all are denuded of epithelium. The majority are crowded especially towards the base with large cells 8.4μ in diameter, containing parasites, which have pushed the nucleus to one side (Plate LXXVIII., Fig. 13). In these parasites nearly all the stages of schizogony, from that of division into four nuclei, can be observed, although rarely in the one field. Plate LXXIX., Fig. 14, shows the various stages as observed, drawn under the camera lucida. The nucleus soon after invasion of the cell by the parasite becomes enlarged and pushed towards the periphery. In the earliest stage of the parasite we have observed, viz., with four nuclei (1.7μ in diameter), the cell nucleus though enlarged is but slightly eccentric. When situated at the very edge, the nucleus may seem at first sight irregular, but careful focussing demonstrates it to be saucer-shaped and clasping the cell. As the parasite grows, the nuclei multiply, and the schizont gradu-

ally fills the cell, which becomes enlarged to three or four times the original diameter, the cytoplasm forming an envelope of the cyst, with the enlarged flattened and elongated cell-nucleus at the edge (Plate LXXIX., Fig. 14). On cessation of multiplication the nuclei gradually become surrounded by protoplasm, and develop into spindle-shaped merozoites (4.3 to 5.5μ long by 2.1 to 2.5μ broad), which, when mature, escape through the attenuated and ruptured cystic cell wall, and may be seen lying free between cells, invading lymph spaces, and even small blood-vessels, and within the lumen of the intestines, these last being the spores observed in the original intestinal smears. Groups of the free spores are to be found within the enlarged and congested mesenteric lymph gland, noted on post-mortem.

As to the nature of the body cells invaded by these two different parasites, it seems to us that in the case of the large cysts they are epithelial in nature, and in the small "cysts" leucocytes. With the large cysts in the absence of the very early stages it is impossible to be dogmatic, but the arrangement and the situation, which is never below the line of gland acini, suggests a simultaneous invasion of a number of epithelial cells in a duct or acinus of Lieberkühn, which would account for the grouping and the peripheral distribution of the cell nuclei. In a very few cases we have observed a single cyst at the edge of a villus which also supports this view.

As in the small "cysts," the cells so definitely invaded are certainly not epithelial, for they are always situated within the basement membrane of the villi. Comparing these cells in the very earliest stages of infection with cells within unaffected villi, and with cells in normal villi, there seems to be no doubt that they are mononuclear leucocytes (Plate LXXVIII., Fig. 13).

Free Spores in Intestine. (Plate LXXIX.).

Smears of the contents of the whole intestinal canal except those of the rectum, especially those smears made from the material immediately covering the mucous membrane, show enormous numbers of spores, evidently those liberated on maturing of the smaller parasites. These spores are much larger than those seen in sections probably owing to the better fixing pre-

venting contraction. The spores are ovoid or spindle-shaped—10.7 to 12.4μ long, by 2.78 to 4.25μ in greatest breadth. Generally one extremity is broader than the other, and stains more deeply. The nucleus is large, stains deeply with iron haematoxylin, and is usually situated towards the broader end of the spore. A few minute granules are to be found, situated chiefly towards the narrower part of the spore.

Ileocystis wombati in Wombat (Phascolomys latifrons).

Unfortunately this animal could not be examined until a lapse of 24 hours after death, consequently the results are not nearly so satisfactory as in the case of the other animals. In view, however, of the pathological and parasitic conditions being similar in many respects to those described in the other native animals, though differing in important details, it is thought they are worthy of record.

The wombat, an adult, had reached Melbourne but a few days prior to death. On exposure of the abdominal viscera the small intestine presented a peculiar reticular mottled appearance. The mucosa on incision was seen to be greatly thickened, the surface being wrinkled in the form of a fairly regular network. On removal of the catarrhal material which covered the surface, this condition was especially noticeable on placing a portion of intestine in water, when the general appearance was that of a thick grey fur.

Only the small intestine was affected, and practically in a uniform manner, the stomach as well as the large intestines being free of any abnormality.

Sections examined microscopically show the pathological changes to be confined to the mucosa and sub-mucosa. The villi are greatly hypertrophied more or less uniformly throughout, but at different points there is much hyperplasia of the underlying connective tissue, giving the wall a papillomatous appearance, and accounting for the reticular, wrinkled conditions so apparent to the the naked eye (Plate LXXX., Fig. 16).

The villi are almost entirely denuded of epithelium, and the great majority of Lieberkühn's crypts are obliterated, only a few of the acini being left. The new connective tissue under

the high power is seen to be distributed in true papillomatous fashion, sending into the lumen of the intestine long processes carrying the hypertrophied villi. These villi in turn are greatly distorted by the presence of large numbers of parasitic cysts (Plate LXXX., Fig. 16), which differ from any of those previously described. These cysts are generally attached to the surface of the villi, which are otherwise denûded of epithelium, but here and there they are to be found crowding and distending the glands of Lieberkühn when they are surrounded by more or less new inflammatory tissue (Plate LXXX., Fig. 17). In none of the sections made from different parts of the intestine are mature cysts seen. On the contrary by far the greater number are evidently degenerated, the contents consisting simply of a granular homogeneously staining material. That this degeneration is not due to the lapse of time after death of the host before the tissue was fixed is evident from the irregular distribution of the living and degenerated cysts (Plate LXXX., Fig. 17, and Plate LXXXVII., Fig. 18). In both cases there is often considerable shrinking of the cystic contents from the wall which is more definite in the degenerated cysts than in the others, and may be to some extent due to fixing. The living cysts which are usually the larger are composed of a thin envelope and an endoplasm, containing many small nuclei (0.85μ to 1.48μ). They are spherical or ellipsoidal, measuring from 0.093 mm. to 0.113 mm. in greatest diameter. The envelope is thin and shows no brush-like arrangement, and in few can any remains of an original cell nucleus be determined. The endoplasm consists of a homogeneous or faintly granular material, staining with eosin and such stains, amongst which in the earliest stages observed are scattered the minute deeply staining nuclei. These nuclei may be arranged without apparent order, but are usually grouped around masses of the protoplasm, and appear as circular, oval, and at times reticulated areas. No further stage of development has been detected in any of our sections.

As the cyst increases in size it should be noted that the nuclei become smaller and more irregularly arranged, so much so that the largest cysts consist of a very thin ectoplasm, containing minute cocci (0.8μ in diameter).

In the earlier stages of the cysts degeneration is much more common than in the later stages, the larger cysts. Here the contents show no nuclei, but simply a granular mass, which stains deeply with eosin, and with the picric acid of Van Gieson stain. The surrounding envelope is thicker in all the earlier stages of the parasite whether degenerated or not, and often a fairly distinct nucleus with definite single nucleolus of the original cell can be observed, both in the degenerated and the living early stages. In the earliest stage noted, the parasite partly degenerated, is situated close to the nucleus, and in the centre of a roughly triangular epithelial cell (45.8μ by 35.4μ) the protoplasm of which completely surrounds the parasite (Plate LXXVII., Fig. 18).

Unhappily, owing to the unavoidable delay in fixing, neither the cell nucleus nor the nucleolus stain definitely or deeply with nuclear stains. It may be therefore that these cysts are not degenerated, but simply a still earlier stage of the parasites' development than that previously described, but at present the evidence is against this supposition.

General.

The large cysts described in the kangaroo, the wombat, and probably also those of the wallaby, although situated beneath the muscularis mucosae, are similar in many respects to the *Gastrocystis gilruthi*, Chatton, 1910, affecting the abomasa of sheep and goats, originally discovered by one of us, and carefully studied by Ed. Chatton. These cysts, which are to be found present in the glandular mucosa of the majority of ovine stomachs, and visible to the naked eye as minute elevations, are composed of an envelope and contents, the latter varying according to the maturity of the parasite (Plate LXXXI., Figs. 19-20). The envelope is formed by a gigantic single cell (parietal cell), and the external surface of the envelope or cell is provided with a uniform very fine brush-like fringe "which ensures an intimate contact and facilitates exchanges with the surrounding tissue." The fringe measures 7 to 9μ in thickness. The flattened and greatly enlarged nucleus of the epithelial cell persists at some part of the periphery and causes a notable thickening of the

wall. The chromatin is spread throughout the nuclear space as granular cords very irregularly anastomosing surrounding the enlarged homogeneous nucleoli.

In mature cysts the contents are formed by innumerable fusiform germs, 10μ by 1.5μ, each of which contains a definitely staining oval nucleus situated near one extremity, and a small centrosome. Generally the nuclear extremity is blunter than the other, which is abruptly narrowed. These germs are arranged radially around a residual mass which gradually disappears as they mature.

In the earlier stages of the cysts observed by Chatton there are three distinct zones; one internal condensed, a median zone of clear cytoplasm, and an external thin zone, formed by the juxtaposition of the roots of the "fringe." The contents consist of a plasmodial mass in which lie numerous glandular nuclei without definite membrane. These nuclei are grouped in divers manners but not without order. Some are arranged as morula, some as large spheres or ellipses, some in groups of two or three or four, etc., but even isolated nuclei may be seen. As the cyst matures the cytoplasmic masses contained in these nuclear morula condense, while the interstitial cytoplasm rarifies. The groups of nuclei then form blastophores, of which all the nuclei are regularly arranged at the surface under a fine membrane or envelope. In all these blastophores simultaneously above each nucleus a small cone of protoplasm appears. These cones increase in size, and gradually form the fusiform germs. In the larger masses all the nuclei do not form germs, a certain number remaining in the residual protoplasm, which itself as well as the interstitial cytoplasm becomes completely absorbed during the maturation of the cysts (Plate LXXXI., Figs. 19-20 c.). It will be seen from this précis of Chatton's work how similar in many respects is the early stage of development of the *Gastrocystis* to that of the large cysts, particularly those of the kangaroo and of the wombat which we have described, and even to some extent of the wallaby cyst.

Chatton discusses the enlargement of the epithelial cells as a result of the invasion by the parasite. He admits that because of its dimensions, its structure, and especially the fringe-like peripheral arrangement it does not appear at first sight a modi-

fied gland cell. He points out that many gregarines and coccidia produce great hypertrophy of affected cells, and so far as the "brush" is concerned recalls that Mrazek maintains this is so in the lymphocytes of *Oligochetes* infected by Microsporidia, and that according to him the myxocystis of which the characteristics are a superficial brush, and two kinds of nuclei, is in reality a complex body formed by a lymphocyte greatly hypertrophied, and by the microsporidium, to the lymphocyte belonging the fundamental mass of the myxocysts, the large nuclei and the peripheral fringe, while to the microsporidium belong the small elements and the spores derived therefrom.

There are many resemblances between the sarcosporidia, and the cysts we have described as well as the *Gastrocystis gilruthi*. The sarcosporidia in the pig (*Sarcocystis miescheri*) also possess a peripheral "fringe," even in the mature cyst. There is also great similarity between the mature merozoites of these parasites and the falciform corpuscles of the sarcocystis, even greater than between the latter and those of the *G. gilruthi*.

Summary.

In this communication what appear to be four varieties of pathogenic neosporidia are described as seen in intestinal affections of three different genera of native fauna.

1. In the wallaby the parasite (*Sarcocystis macropodis*) is situated in the submucosa, and is surrounded by chronic inflammatory tissue. It is composed of an envelope with fringed periphery, and an endoplasm separating the blastophores, which in the earlier stages are composed of nuclei arranged peripherally around masses of protoplasm, and in the later stages of mature spores, similar to those of sarcosporidia. No nucleus of an original tissue cell can be detected. But assuming the cyst to be derived from an invaded body cell greatly hypertrophied, this cell does not appear likely to be epithelial in character, but probably a lymph or endothelial corpuscle. As in *Balbiania* the origin may be a muscle fibre, but the sections do not support such a view.

2. In the kangaroo there are apparently two parasites. The larger *Ileocystis macropodis* are generally present in groups. Each possesses an envelope with a regular fringe at the periphery, and a small

amount of endoplasm surrounding innumerable nuclei, which in the earlier stages are irregularly distributed, but in the later stages are arranged around masses of protoplasm. Within the endoplasm, lying close to the envelope of each cyst, at the pole furthest from its neighbours, lies an irregularly spherical, oval or crescentic deeply-staining granular body, which is probably the greatly hypertrophied nucleus of the invaded cell. The majority of these large cysts are situated at the base of a villus, but occasionally some may be seen (generally singly) nearer the apex, situated always at one side of the villus. Assuming that these represent greatly hypertrophied body cells the indications are that they are epithelial in type.

The smaller "cysts" (*Lymphocystis macropodis*) are situated underneath the basement membrane of the mucosa, and are definitely formed within invaded cells, probably mononuclear lymph cells. The cell-nucleus, even in the very early stages, when the parasite consists of but four small nuclei surrounded by a faintly staining spherical mass of protoplasm, is distinctly pushed to the side of the cell, and becomes hypertrophied as the cell increases in size owing to the growth of the parasite, ultimately becoming saucer-shaped, clasping the periphery of the cell. The nuclei of the parasite multiply greatly, do not become regularly arranged, and finally form falciform spores, which escape from the mature cyst into the lumen of the intestine on the one hand, and into the lymph stream on the other, as a result of which they may be found in adjacent lymph glands.

3. In the wombat (*Ileocystis wombati*) the cysts are arranged around the periphery of the villi, and within the glands of Lieberkühn. They are present in enormous numbers, and induce a papillomatous condition of the mucosa similar to severe coccidial infection. The majority of the glands are completely obliterated by the infection and by the tissue reaction, while the villi are enormously hypertrophied. Many of the smaller cysts appear to be degenerating, and these show a definite envelope, frequently containing an enlarged cell nucleus, with single spherical nucleolus. The living cysts are usually much larger, have a thin envelope, with seldom any definite nuclear body, and an endoplasm containing in the early stages irregularly-distributed minute nuclei, which, as the parasite develops, become arranged

in more or less spherical mulberry groups, around masses of endoplasm. That here we have an invasion of the epithelia there is little reason to doubt. Unfortunately no fully-developed spores can be found, so that the mature stage of the parasite remains unstudied.

These parasitic protozoa affecting the intestines of different Australian native animals appear to be of species hitherto undescribed.

Judging from the appearance and the situations especially, probably four new species have been encountered. That they can hardly be included with the *Sarcosporidia*, is evident from their situation, and from their general structure. There is no doubt that even the wallaby cysts have not their commencement in muscle cells, for they, in the early stages, are seen to be quite apart from any of the muscle coats. The kangaroo large cysts are probably within epithelial cells, while the smaller cysts are definitely within mononuclear leucocytes. The wombat cysts are undoubtedly in epithelial cells. The nature of the cysts is in no case similar to the sarcosporidium. Each may, however, be included in the same class *Neosporidia*.

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DESCRIPTION OF PLATES.

Plate LXXII.

Fig. 1.—Wallaby cyst. Almost mature stage, showing irregularity of fringe and distortion of cyst with surrounding inflammatory reaction and situation. (Drawn by aid of camera lucida.)

Fig. 2.—Wallaby cyst, early stage. (Drawn by aid of camera lucida.)

PLATE LXXIII.

Fig. 3.—Wallaby. Oblique longitudinal section, showing groups of cysts in various stages of development, with surrounding inflammatory tissue. (*a*) muscle coats, (*b*) mucosa, (*c*) groups of cysts, (*d*) inflammatory tissue. (Photomicrograph × 18).

Fig. 4.—Wallaby. Transverse section of intestine, showing many cysts. (Photomicrograph × 18).

PLATE LXXIV.

Fig. 5.—Wallaby. Edge of young cyst, showing regular fringe, endoplasm and blastophores. (Photomicrograph × 420).

Fig. 6.—Wallaby. Edge of mature cyst, showing very irregular fringe, with atrophy of muscular and mucous coats. (Photomicrograph × 460).

PLATE LXXV.

Fig. 7.—Wallaby cyst. Blastophore, showing mature spores with masses of residual protoplasm. The radial arrangements of the spores has disappeared, and the masses of residual protoplasm are unusually large. (Drawn by the aid of camera lucida.)

Fig. 8.—Wallaby. Mature spores of parasitic cysts, drawn from smears. (Drawn by aid of camera lucida.)

PLATE LXXVI.

Fig. 9.—Kangaroo. Section showing two groups of large cysts. (*a*) early stage, (*b*) stage of almost maturity, showing hypertrophied and cresentic cell-nuclei at periphery (*c*) groups of small "cysts" in vicinity. (Photomicrograph × 110).

Fig. 10.—Kangaroo. Groups of large cysts, showing hypertrophied cell nuclei. (Photomicrograph × 340).

Plate LXXVII.

Fig. 11.—Kangaroo. Section of intestine, showing—(*a*) groups of large cysts, and (*b*) hypertrophoid villi. (Photomicrograph × 70).

Fig. 18.—Wombat. Section showing—(*a*) degenerating cysts, (*b*) cell nucleus, (*c*) large living cyst, (*d*) unaffected crypts of Lieberkühn. (Photomicrograph × 370).

Plate LXXVIII.

Fig. 12.—Kangaroo. From section: group of hypertrophied cells invaded by large parasites—(*a*) cell showing nucleus and parasite, (*b*) cell showing parasite, but no nucleus, (*c*) cell showing nucleus, but no parasite, (*d*) cell showing neither nucleus nor parasite (*e*) fringe. (Drawn by aid of camera lucida.)

Fig. 13.—Kangaroo. Cross section of villus showing schizonts within mononuclear lymph cells. (Drawn by aid of camera lucida.)

Plate LXXIX.

Fig. 14.—Kangaroo. Development of small schizonts within monoculear lymph cells. (Drawn by aid of camera lucida.)

Fig. 15.—Kangaroo. Mature spores of small cysts. (Drawn by aid of camera lucida.)

Plate LXXX.

Fig. 16.—Wombat. Section of intestine showing papillomatous condition with parasitic cysts in mucosa. (Photomicrograph × 18).

Fig. 17.—Wombat. Section of mucosa, showing—(*a*) large living cysts, (*b*) smaller degenerating cysts, (*c*) unaffected crypts of Lieberkühn. (Photomicrograph × 90).

Plate LXXXI.

Fig. 19.—Wombat. Degenerating schizonts within epithelial cells. (Drawn by aid of camera lucida.)

Fig. 20.—*Gastrocystis gilruthi*, Chatton.

A. Within mucosa of abomasum of sheep. Plasmodial stage before spore formation, while zone around the cyst represents the fringe. At the right is situated the hypertrophied nucleus of the invaded parietal cell.

B. Mature spores.

C. Formation of spores of a blastophore. (After Chatton.)